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Effects of Halothane Anesthesia on Blood Cholinesterase Activity in Cats

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The effect of halothane anesthesia on blood cholinesterase activity was assessed in 24 adult cats. Blood samples were taken both before and during the course of halothane anesthesia. Acetylcholinesterase activity was depressed from 7 percent to 54 percent (average 19.5 percent) in 16 subjects, increased in 2 (9 percent and 11 percent), and was unchanged in the other 6. The mean acetylcholinesterase change for the entire population was a 12.3 percent decrease. Pseudocholinesterase (butyrylcholinesterase) activity was depressed from 7 percent to 24 percent (average 12.2 percent) in 19 subjects, and was unchanged in the other 5. The mean butyrylcholinesterase depression for the entire population was 11 percent. There was no apparent correlation between the weight or gender of the animal, or the length of time on halothane, and the amount of depression in cholinesterase activity. Neither was there close agreement between changes in acetylcholinesterase and butyrylcholinesterase activity in a single cat. These results demonstrate that halothane has an inhibitory effect on blood cholinesterase activity in many cats (96 percent of

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those tested when counting either acetyl- or butyrylcholinesterase). The lack of agreement between changes in acetylcholinesterase and butyrylcholinesterase activity in the same animal suggests that the mechanisms may be different. It remains to be determined whether the amount of enzyme inhibition following halothane is functionally significant.

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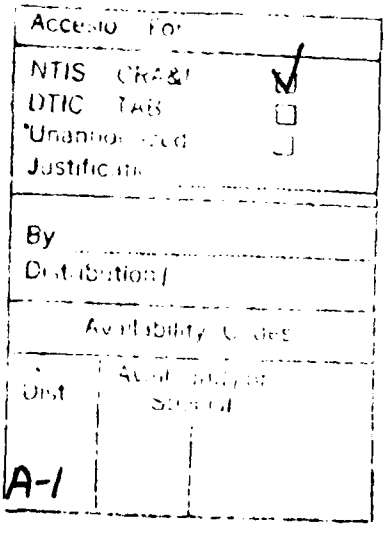

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Introduction

Halothane is used widely as a general anesthetic in both animal and human surgery. During studies investigating cholinergic involvement in the visual pathway of the cat (Harding, Kirby, and Wiley, 1985; Kirby, Harding, and Wiley, 1987), it was noted that there was an apparent decrease in blood cholinesterase activity during halothane anesthesia (Kirby et al., 1985). Unfortunately, base line cholinesterase levels for most cats in those studies were determined from blood samples obtained when the animals first entered the colony. The reported decrease in blood cholinesterase therefore could result from changes occurring in the cat colony rather than from exposure to halothane.

Activity of synaptically released acetylcholine (ACh) is terminated by the degradative enzyme acetylcholinesterase (AChE), or true cholinesterase, which hydrolyzes ACh to choline and acetic acid. AChE is found in neurons and on red blood cells. There is a second class of cholinesterase found in living systems, pseudocholinesterase (butyrylcholinesterase; BuChE), which preferentially hydrolyzes higher choline esters, and is found in nerve tissue as well as in blood plasma. If halothane depresses cholinesterase activity, studies investigating neural function during or after halothane anesthesia actually could be assessing the effect of excess ACh. We undertook the current study to investigate more directly the possible effect of halothane on blood cholinesterase activity (both AChE and BuChE) in the cat.

Materials and methods

Initially, 24 adult cats (2.4-7.4 kg) of either sex were restrained manually while a sample of venous blood was drawn from the jugular or cephalic vein. Anesthesia then was induced with 3 to 4 percent halothane in a 3:1 gas mixture of nitrous oxide and carbogen, and maintained with 1 to 2 percent halothane in the same gas mixture. After 30 minutes or more of halothane anesthesia, one or more additional blood samples were obtained. The determination of AChE and BuChE activity in blood was done according to a colorimetric assay procedure (Ellman et al., 1961). Briefly, for AChE a suspension of blood cells was prepared in phosphate buffer (pH 8.0) at a dilution of 1:600. A 1:600 dilution of plasma in buffer was prepared for BuChE. Three ml of the suspension was pipetted into a cuvette along with 25 µl of dithiobisnitrobenzoic acid (DTNB) and 20 µl of the substrate, acetylthiocholine iodide or butyrylthiocholine iodide. Enzyme activity then was measured photometrically by following the increase in absorption (measured at 412 nm) produced from thiocholine reacting with the DTNB ion. Results are expressed in terms of moles of substrate hydrolyzed/min/red blood cell for AChE or per µl plasma for BuChE. Based upon repeated

determinations of enzyme activity from the same sample of blood, our measurement error was taken to be ±5 percent. Some animals were allowed to awaken from the anesthesia, but most were used in different experiments during which the halothane was removed from the gas mixture and their cholinesterase activity followed over longer time periods under various conditions.

Results

Results from all 24 cats are presented in Table 1. It shows the gender and weight of each animal, the blood AChE and BuChE activity both pre- and during halothane anesthesia, and the time each animal was exposed to halothane before the second blood sample was taken. Two of the animals demonstrated increased AChE activity (9 and 11 percent), 6 showed essentially no change, and the other 16 showed decreased AChE activity during halothane anesthesia (7 to 54 percent reduction). The average for all 24 cats was a 12.3 percent reduction. As a population, the difference in AChE activity between awake and halothane-anesthetized animals was significant ($p < 0.01$, matched-pair t-test). Although the sample was skewed heavily in favor of females, there was no apparent connection between the gender or weight of the animal or the length of time exposed to halothane, and the direction or magnitude of change in AChE activity.

Table 1.

Blood AChE and BuChE activity for 24 cats

Cat #	Sex	Wt. (Kg)	AChE/Awake	AChE/ Halothane	BuChE/ Awake	BuChE/ Halothane	Hrs. on Halothane
3482	F	2.5	3.468	3.590	1.949	1.657	2.0
3035	M	5.5	1.921	1.556	1.266	1.158	0.5
55	M	7.4	1.143	1.244	1.167	1.078	0.5
57	M	6.2	1.683	1.136	1.625	1.329	0.5
58	F	2.8	1.164	1.162	1.289	1.149	1.5
59	M	5.5	1.422	1.199	0.889	0.763	0.5
61	F	3.6	2.882	2.765	2.119	1.926	0.5
63	F	2.6	1.470	1.165	1.428	1.307	0.5
65	F	2.7	1.735	1.528	1.360	1.172	1.5
66	F	3.0	1.904	1.595	1.316	1.158	3.3
67	F	3.2	1.700	1.651	1.365	1.217	1.8
71	F	3.3	2.391	2.156	1.666	1.155	2.0
72	F	2.4	3.040	1.410	1.661	1.257	2.3
73	F	3.6	1.442	1.338	1.392	1.387	2.8
74	F	3.4	2.609	2.523	1.688	1.598	2.5
75	F	2.6	2.066	1.739	1.347	1.275	2.3
76	F	2.6	2.027	1.892	1.724	1.473	2.8
77	F	2.6	3.942	4.081	1.774	1.486	2.0
78	F	2.4	1.900	2.100	1.688	1.351	2.8
80	F	3.6	1.469	1.267	1.145	1.046	1.8
81	F	3.8	1.819	1.664	1.585	1.387	2.3
84	F	3.2	1.360	0.970	1.230	1.253	1.5
86	M	4.4	2.140	1.490	1.792	1.666	1.8
90	M	3.6	1.230	1.010	1.266	1.221	1.8

AChE activity in moles hydrolyzed/min/RBC ($x10^{-16}$)

BuChE activity in moles hydrolyzed/min/µl plasma ($x10^{-10}$)

Nineteen of the 24 cats showed a decrease in BuChE activity during halothane anesthesia (7 to 24 percent reduction). The other 5 animals showed no change. The average for all 24 cats was an 11 percent reduction. As a population, the difference in BuChE activity between awake and halothane-anesthetized animals was significant ($p < 0.001$, matched-pair t-test). As with AChE, there was no connection between change of BuCnE activity and the gender or weight of the animal, or the length of time exposed to halothane. Although cat #72 demonstrated the greatest decrease in both AChE and BuChE activity, only one of the six animals showing no change in AChE activity also showed no change in BuChE activity. The others all demonstrated decreased activity. Four of the five cats demonstrating unchanged BuChE activity during halothane showed decreased AChE activity.

The AChE data from Table 1 are presented graphically in Figure 1, where the prehalothane AChE activity is plotted against the AChE activity determined during halothane anesthesia for the

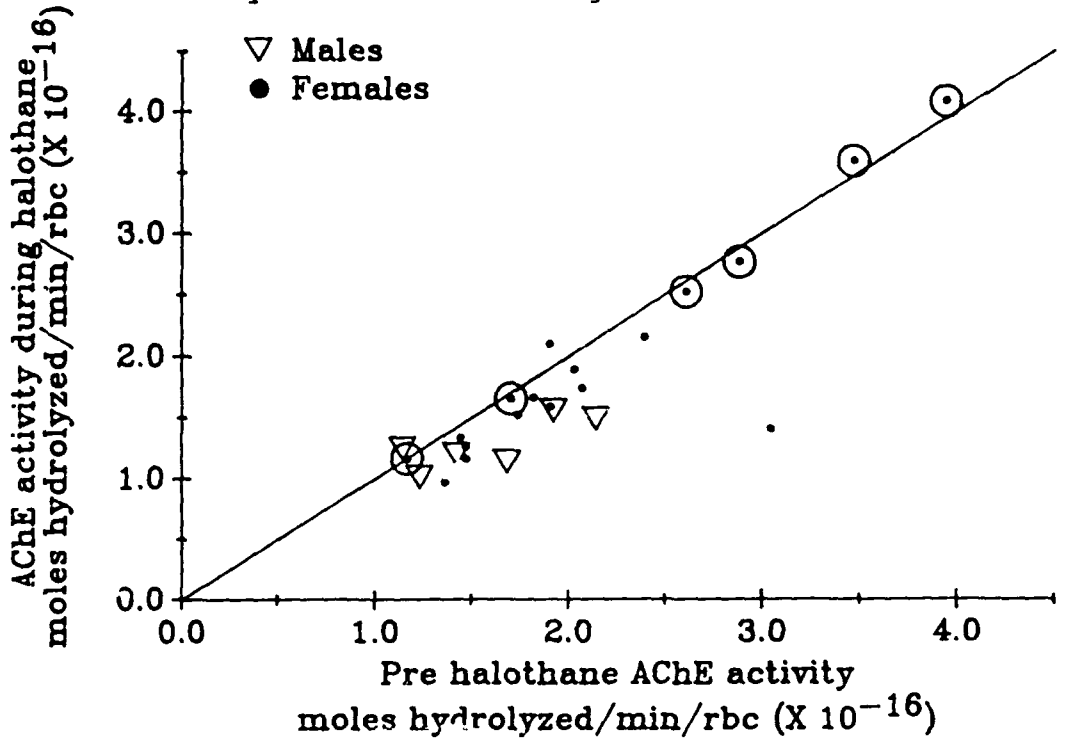

Figure 1. Prehalothane blood AChE activity plotted against AChE activity during halothane anesthesia for the 24 cats listed in Table 1. The AChE activity is depressed during halothane in 16 of the 24. The difference indicated by the six circled points is within our measurement error. The line of slope 1 is drawn only as a comparison; animals plotted above the line showed increased AChE activity while those below the line showed decreased activity.

24 cats. A line of slope 1 has been drawn from the origin. Any values for AChE activity which have increased during halothane would fall above the line (1 male and 1 female), while decreased AChE activity during halothane would be plotted below the line. Note that the AChE activity is depressed following halothane in 16 of the 24 cats.

The prehalothane BuChE data from Table 1 is plotted against the BuChE values determined during halothane anesthesia in Figure 2. As with the AChE values in Figure 1, decreased BuChE activity during halothane would be plotted below the line of slope 1. It is quite clear that most of the points fall below the line. The difference indicated by the five circled points is within our measurement error.

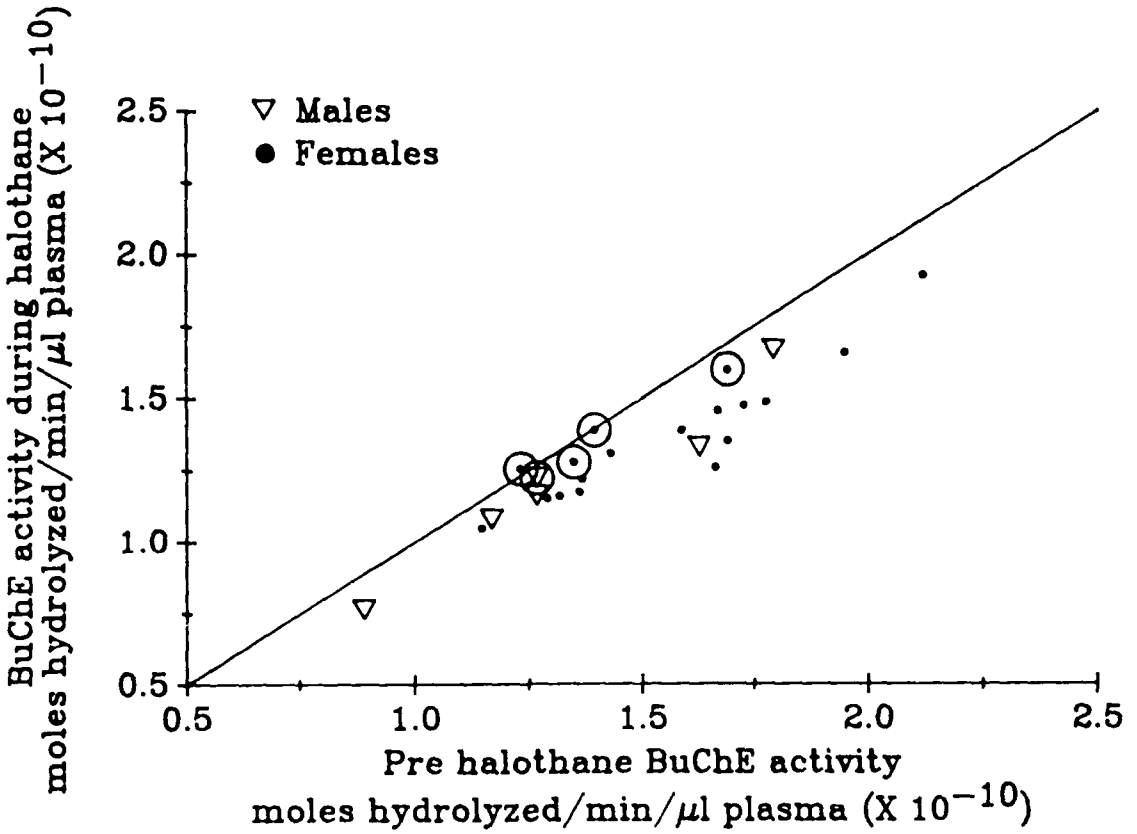

Figure 2. Prehalothane plasma BuChE activity plotted against BuChE activity during halothane anesthesia for the 24 cats listed in Table 1. The BuChE activity is depressed during halothane in 19 of the 24 cats. The difference indicatedby the five circled points is within our measurement error. The line of slope 1 is drawn only as a comparison; animals plotted above the line showed increased BuChE activity while those below the line showed decreased activity.

Figure 3 shows reduction in blood cholinesterase activity for a single cat during halothane anesthesia and recovery to base line levels following cessation of halothane for AChE. The BuChE activity showed no tendency for recovery. The cat was exposed to halothane for 3.25 hours during which time AChE activity was reduced 18 percent and the BuChE activity reduced 22 percent. After 4 hours without halothane, the AChE activity had returned to within 3 percent of the prehalothane level, while the BuChE activity still was inhibited 20 percent. The animal then was used in another experiment. Similar results were obtained from four other animals with recovery of AChE activity ranging from 3 to 6 hours after removal of halothane from the gas mixture (mean 4.8 hrs $\pm$ 1.3hrs; n=5). Only one of the five animals showed any trend for recovery in BuChE. It was exposed to halothane for almost 4 hours. The BuChE activity was reduced 14 percent during that time, and had recovered to within 5 percent of base line by 3 hours after halothane.

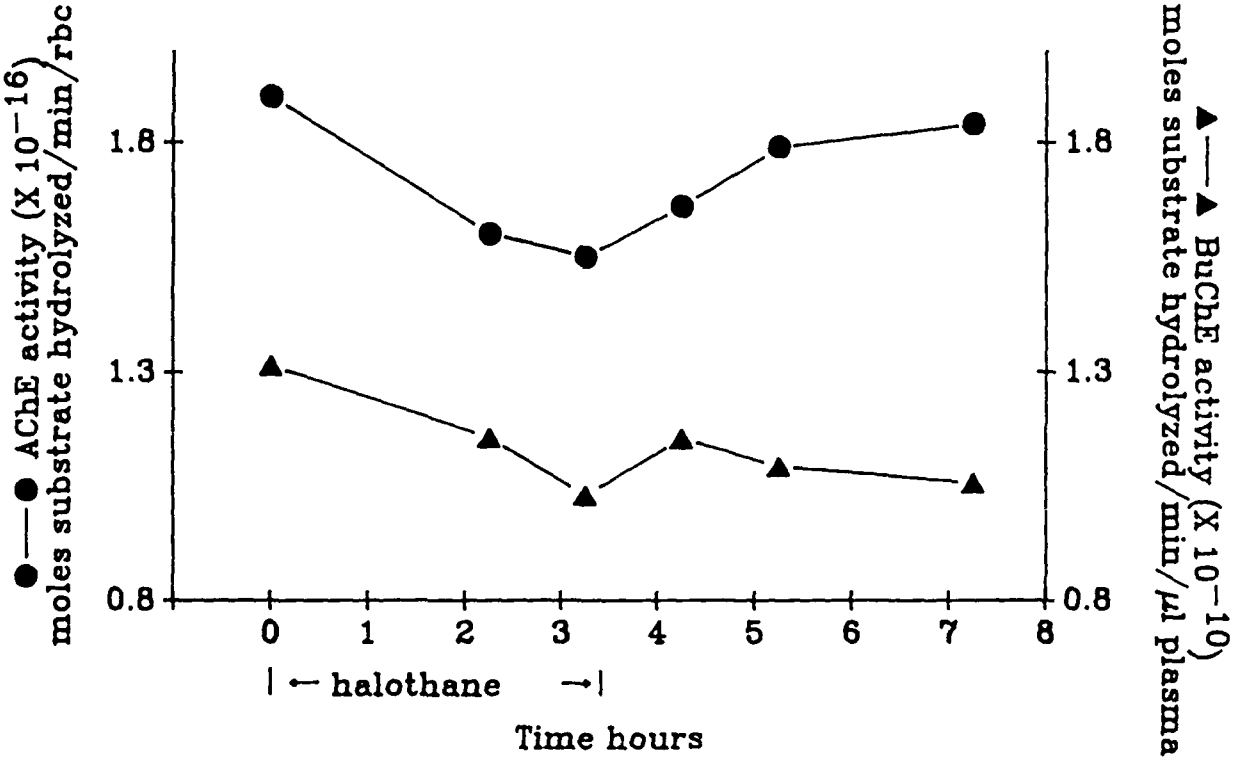

Figure 3. Blood AChE and plasma BuChE activity plotted against time for a single cat exposed to halothane for 3.25 hours. AChE activity was reduced 18 percent while BuChE activity was reduced 22 percent. After 4 hours without halothane, the AChE activity had returned to within 3 percent of base line while BuChE activity showed no tendency for recovery. The animal then was used in another study.

Discussion

The results show that halothane in the concentrations used depresses blood cholinesterase activity, either AChE or BuChE, in most of the cats tested (96 percent, n=24). The effect on AChE is reversible fully once halothane is discontinued, while BuChE activity shows a much greater tendency to remain inhibited. Since the animals from which the data of this report were collected were scheduled for other studies, we have no information on possible long-term recovery of BuChE activity.

The amount of cholinesterase inhibition shows no apparent correlation with the gender or weight of the animal or the length of halothane exposure. Although the 6 cats reported here showing no change in AChE activity during halothane exposure were females, it should be emphasized that our experimental sample was heavily skewed in that direction (18 females out of 24 cats). Five of the six animals showing no change in AChE did show reduction in BuChE activity. We have no information as to the mechanism of cholinesterase inhibition by halothane, but it appears to differ for AChE and BuChE judging from recovery, since in a single animal we see recovery of AChE, but not BuChE activity following halothane. Similar findings of reductions in cholinesterase activity in cats following halothane anesthesia recently have been brought to our attention (Alistair Webb, personal communication).

There is certainly tremendous interest concerning the role of acetylcholine as a central neurotransmitter and its possible interaction with other neurotransmitter systems. Since the levels of synaptically released ACh are regulated by the action of cholinesterase, and halothane inhibits both AChE and BuChE in the blood, use of halothane anesthesia certainly could alter central function. We have no direct evidence from these studies whether brain cholinesterase actually is inhibited by halothane, since all the animals were used in other studies following collection of blood samples. However, we previously have seen a close relationship between cortical and blood cholinesterase in several cats (unpublished observation), and a close correlation between plasma and brain cholinesterase activity has been reported in the rat following administration of an anticholinesterase agent (Shih, 1983).

If we assume that brain cholinesterase is inhibited by halothane, the question arises as to whether enough AChE might be nhibited to cause a functionally significant increase in the amount of synaptic ACh. There is some evidence in the literature to support that hypothesis. Increasing alveolar halothane concentration in humans results in an increased latency in the P1 peak of the cortical visual evoked potential (Uhl et al., 1980), and halothane anesthesia in rabbits affects latencies, ampli-

tudes, and wave shape of the visual evoked response (VER) (Gerritsen, 1970). Since administration of anticholinesterase agents alters the VER in cats (Harding, Kirby, and Wiley, 1985; Harding, Wiley, and Kirby, 1983), and nonhuman primates (Woolley, 1976), the VER changes during halothane anesthesia (Uhl et al., 1980; Gerritsen, 1970) could result from cholinergic inhibition.

Until recently, BuChE was reported to only a limited extent in neuronal tissue (Mayer, 1980). Recently, however, the distributions of AChE and BuChE were compared in the central visual pathway, and the histochemical localization of BuChE was shown to rival that of AChE (Graybiel and Ragsdale, 1982). This suggests that the depression of BuChE activity by halothane certainly could have functional significance as well, especially in the visual system. Since we did not use a specific inhibitor of BuChE when assaying for AChE, or a specific inhibitor of AChE when assaying for BuChE, the values obtained for the activities of the two enzymes are likely not entirely independent of each other. However, substantial independence of the two activities is demonstrated when AChE, but not BuChE, activity returns to base line following cessation of halothane. It does seem safe to conclude that halothane inhibits cholinesterase activity in the cat. Investigators utilizing halothane in their preparation must therefore be concerned that the cholinergic system may well be hyperactive in their animals.

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